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Irradiation and storage influence on bioactive components and quality of early and late season 'Rio Red' grapefruit (*Citrus paradisi* Macf.)

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Abstract

Irradiation as an alternative quarantine treatment has been under consideration by the International Consultative Group on Food Irradiation. This study was conducted on early and late season 'Rio Red' grapefruit to investigate the effects of harvest date, storage, and low dose irradiation on functional constituents, and quality. Fruit was treated with 0, 70, 200, 400 and 700 Gy and then stored under simulated storage conditions by subjecting the fruit to 10 °C for 4 weeks followed by 1 week at 20 °C with 90–95% relative humidity. Flavanones (naringin and narirutin), terpenoids (limonin 17-β-D-glycopyranoside, β-carotene and lycopene) and quality (ascorbic acid content, soluble solids (%), titratable acidity) were evaluated immediately following irradiation treatment and storage. Results demonstrated that the response of fruit to irradiation depended on harvest time. Lower doses (at or below 200 Gy) of irradiation coupled with 35 days of storage were useful in enhancing health promoting compounds in early season grapefruit. Higher doses of irradiation (400 and 700 Gy) and 35 days of storage had detrimental effects on quality of early season grapefruit, however, no significant effect was observed on the quality of the late season fruit.
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Keywords: Gamma irradiation; Storage; Quality; Grapefruit; Health; Functional components

1. Introduction

Texas grapefruit exported to international markets such as Japan or domestic markets such as Florida, California and Arizona must be certified free of Mexican fruit fly, *Anastrepha ludens* (Loew). Currently, methyl bromide (MB) fumigation is one of the commercial quarantine treatments used in Texas to overcome trade barriers; however, it is toxic to humans and causes damage to the stratospheric ozone layer. Although exemptions to use methyl bromide for post-harvest treatments have been granted, it is anticipated to be phased-out by the year 2010 in developed countries. The international community has also strongly sensed that an alternative treatment must be developed before the total phase out of MB. The United Nations Environmental Programme (UNEP) established the Methyl Bromide Technical

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Options Committee (MTOC), which has identified irradiation as being a versatile and viable alternative for quarantine treatment in the fight against fruit flies.

A low dose of gamma irradiation as a quarantine treatment against Mexican fruit fly was recently developed for citrus fruit. Recent studies in our laboratory have shown that a minimum dose of 58 or 69 Gy was suggested for disinfestations; however, depending on the level of security required in commercial scale operations, fruit could receive up to three times the minimum absorbed dose for disinfestations (Hallman and Martinez, 2001).

Treatment of grapefruit with a dose of 300 Gy resulted in minimal injury to the fruit (Spalding and Davis, 1985; Miller and McDonald, 1996). Furthermore, our studies have demonstrated that 'Rio Red' grapefruit exposed to irradiation doses of up to 500 Gy did not affect soluble solids (%), titratable acidity, appearance, and organoleptic quality compared to untreated fruit (Hallman and Martinez, 2001).

Type and intensity of injury to grapefruit due to low dose irradiation (300–900 Gy) has been attributed to time of harvest. Early-season grapefruit, harvested from October to December, were more susceptible to scald and less susceptible to rind breakdown, while late-season fruit were more susceptible to rind breakdown after irradiation and storage (Hatton et al., 1982). Irradiation applications to improve bioactive components in fruit and vegetables has been reviewed (Patil, 2004).

Although studies have reported the effect of low dose irradiation on grapefruit quality parameters such as soluble solids, acidity and appearance, very little information is available on the effect of low dose irradiation on health promoting compounds in grapefruit such as flavanones (naringin and narirutin), limonin, carotenoids, lycopene and Vitamin C. The present study was undertaken to examine the effects of gamma irradiation and simulated storage conditions on functional constituents and quality of grapefruit harvested in different seasons.

2. Material and methods

'Rio Red' grapefruit were collected from an orchard at the Texas A&M University-Kingsville Citrus Center South Farm, and fruit were run in a commercial packing line, washed and waxed.

Fruit were irradiated with ^{137}Cs self-contained dry-storage irradiators (Husman Model 521A, Isomedix, Inc., Whippany, NJ) at the USDA facility in Mission, TX. Sixteen fruit per treatment were exposed to 0, 70, 200, 400, and 700 Gy with a centerline absorbed dose of about 40 Gy min^{-1}. After the irradiation treatment, fruit were subjected to simulated storage conditions by storing for 4 weeks at 10 °C followed by one week at 20 °C with 90–95% relative humidity. Fruit from each treatment was collected for extraction and analysis of phytochemical levels both 24 h after irradiation and at the end of storage. Samples were stored at −80 °C until analyzed.

2.1. Laboratory analysis

Fruit samples were analyzed for flavanone content, limonin glucoside, and Vitamin C. Fruit quality parameters such as soluble solids (%) using temperature controlled refractometer and titratable acidity (TA) was determined by titration of juice with 0.1N sodium hydroxide to phenolphthalein endpoint (expressed as mM H^+). The soluble solids/acid ratio was calculated from two values (soluble solids/TA).

2.2. Standards

Naringin and ascorbic acid standards were purchased from Sigma Chemical Co. St. Louis, MO, USA. Limonin 17-β-D-glucopyranoside (LG) was purified according to our established procedure (Tian et al., 2001). Narirutin was supplied by John Manthey, USDA-ARS, Winter Haven, FL.

2.3. Flavanone and limonin 17-β-D-glycopyranoside analysis

Samples were analyzed according to Berhow (2000) for flavanone content by reverse phase liquid chromatography with some modifications. An aliquot of juice was diluted 1:1 with dimethylsulfoxide, and subsequently centrifuged and filtered though a 0.45 μm nylon filter. Twenty microliters of solution was injected into the HPLC system. Separation of flavonoid compounds was performed using a stainless-steel Adsorbosil C-18 column (250 × 4.6 mm i.d.) and

a solvent system of acetonitrile (ACN)/water plus 5 mM phosphoric acid starting at 10% and ending at 26% ACN concentration. The narirutin and naringin peaks were detected at 280 ηm at retention times of 25 and 27 min, respectively. The flavanones were identified by matching their respective spectra and retention times with those of commercially obtained standards. Extraction and quantification methods used for flavanones were employed for limonin 17-β-D-glycopyranoside; however, it was detected at 210 ηm with a retention time of 47 min.

Peak areas were normalized to the external standard and a standard curve was fitted by linear regression (peak areas versus concentration in mg kg^{-1}). Total flavanones were calculated by combining naringin and narirutin concentrations.

2.4. Vitamin C analysis

One ml of juice was homogenized with 3 ml of citric acid (3%). An aliquot of 0.8 ml was centrifuged at 4000 min^{-1} for 20 min and filtered through a 0.45 μm nylon filter (Alltech Associates, Deerfield, IL). Twenty microliters of this solution was injected into the HPLC system. A Waters Bondpak-C-18 column (30 × 0.4 cm) with a guard column was used for separation. The mobile phase contained acetonitrile: water (70:30, v/v) with 0.01M ammonium phosphoric acid at the flow rate of 1.5 ml min^{-1}. Vitamin C was detected at 255 ηm with a retention time of 6 min. Soluble solids (%) and titratable acidity were measured using standard practices.

2.5. Consumer preference evaluation

During both early and late season, fruit were evaluated for consumer acceptability by 10–12 average untrained panelists (Texas A&M University-Kingsville Citrus Center). Preference evaluation was conducted to determine the flavor and external appearance of the fruit both during initial (0 day of the treatment) and final evaluation (after 35 days of simulated storage conditions as explained above). Quantitative preference rating (American Society for Testing and Materials, 1968) was used to evaluate ratings for flavor and external appearance for irradiated and control fruit. Eight slices were prepared from each fruit and four slices (randomly selected from five fruit) from each treatment were presented for flavor analysis. We also presented whole 'Rio Red' grapefruit (five fruit) that were included for appearance evaluations. Separate booths were used to conduct preference evaluations. Hedonic scale ranging from extremely dislike to extremely liking (1–9) were used by judges to indicate their preferences through vertical lines.

2.6. Statistical analysis

The data was analyzed using a 5 × 2 factorial design with irradiation dose and storage as factors (GLM procedures, SAS Institute Inc., Cary, NC). Tukey's test was employed for all mean separation analyses.

3. Results and discussion

3.1. Changes in naringin, narirutin and total flavanones

Irradiation and low-temperature storage significantly affected the flavanone content of grapefruit. In general, the early season grapefruit exposed to low doses of irradiation (70 and 200 Gy) followed by storage (35 days) had significantly ($P \leq 0.05$) higher naringin (Fig. 1), narirutin (Fig. 2) and total flavanone concentrations (Fig. 3) compared to the initial (0 day) flavanone concentrations. This increase may be attributed to an increase in phenylalanine ammonia lyase (PAL) activity during low temperature storage (Faragher, 1983) and low dose irradiation exposure (Oufedjikh et al., 2000). Fruit exposed to 200 Gy irradiation, after the simulated storage conditions, had numerically higher total flavanone content compared to other treatments. Interestingly, an increase in irradiation dose resulted in decrease of naringin, narirutin, and total flavanone content immediately after irradiation. Furthermore, decrease in flavanone contents was more evident at higher doses (400 and 700 Gy). Our results are consistent with reports of Oufedjikh et al. (1996). The same authors also reported that the concentration of flavanone glucosides and polymethoxylated flavones were significantly lower in irradiated fruit (300 Gy) at 0 day of storage. The decrease in flavanone content was ascribed to their role in counteracting the oxidative stress induced by the gamma irradiation. Variations in the flavanone content

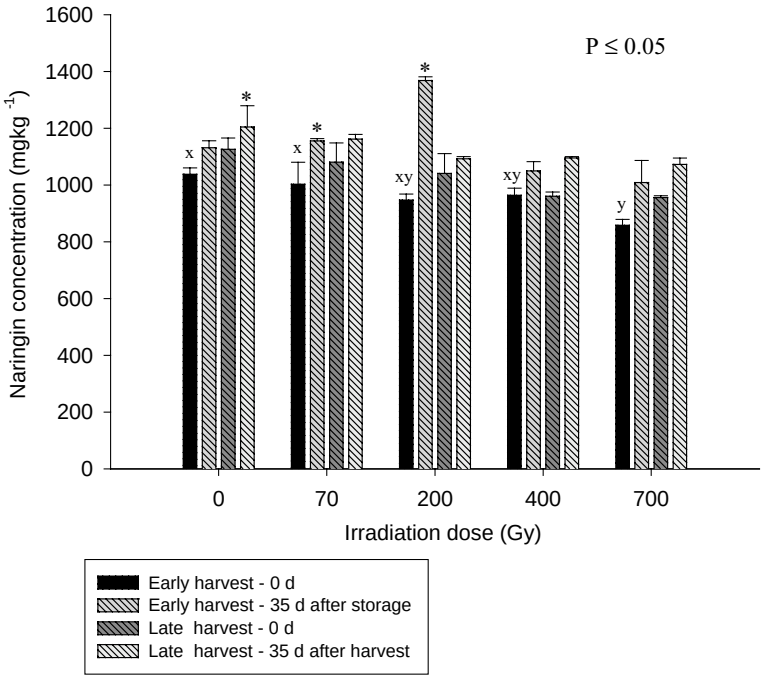

Fig. 1. Irradiation and storage effects on naringin content of early and late season 'Rio Red' grapefruit. Asterisk indicates significant ($P \leq 0.05$) differences between the mean values of 0 and 35 days after storage for early harvest fruit. Same letter on the bar for early harvest (0 day) fruit indicates no significant differences at $P \leq 0.05$.

at different doses of irradiation treatment may be due to equilibrium between gamma irradiation induced oxidative stress and de novo synthesis of flavonoids by increased PAL activity (Oufedjikh et al., 1996).

Our results seem to suggest that gamma irradiation had a differential effect on early and late harvest grapefruit. Non-irradiated (0 Gy) grapefruit showed significantly higher concentrations of narirutin as compared to fruit irradiated at or above 200 Gy in early harvest fruit at 0 day of storage. Total flavanone content was significantly higher in late season non-radiated fruit compared to irradiated fruit after the 35 days of storage. Interestingly, irradiation had no significant ($P \leq 0.05$) effect on naringin content of late season grapefruit. In general, flavanone concentrations were decreased with increasing irradiation dose even in the late season grapefruit, and storage had a positive effect on flavanone concentrations. The observed variations in the concentrations of flavanones at similar irradiation doses from different seasons could be attributed to the differences in climatic conditions (ex. higher temperature at the late season) and differences in maturity/senescence of the fruit.

3.2. Changes in terpenoids (β-carotene, lycopene, total carotenoids and limonin glucoside)

In both early and late season, applied doses of irradiation and 35 days of storage affected the terpenoid content (Figs. 4–7). While both irradition and storage influenced terpenoid content of grapefruit, the storage effect was more pronounced than the irradiation effect. Irrespective of the irradiation treatment, early season grapefruit had significantly ($P \leq 0.05$) higher levels of β-carotene after the 35 days of storage conditions than their initial (0 day) level (Fig. 4). On the contrary, in late season fruit, irradiation had no effect on the β-carotene content of grapefruit before or after 35 days of storage conditions. Sebastiao et al. (2002) reported that gamma irradiation doses of 0, 10 and 20 kGy neither affected β-carotene nor contributed to the decrease of Vitamin A. Late season fruit treated

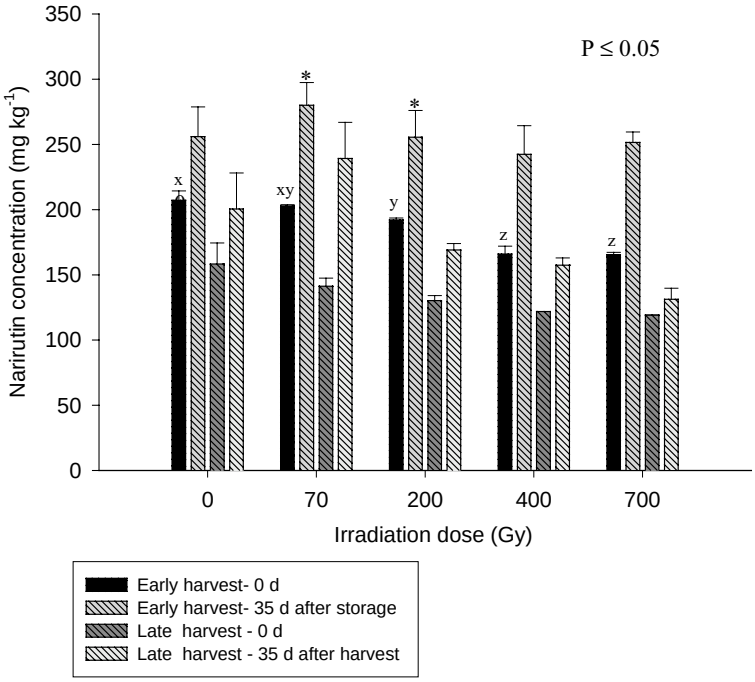

Fig. 2. Irradiation and storage effects on narirutin content of early and late season 'Rio Red' grapefruit. Asterisk indicates significant ($P \leq 0.05$) differences between the mean values of 0 and 35 days after storage for early harvest fruit. Same letter on the bar for early harvest (0 day) fruit indicates no significant differences at $P \leq 0.05$.

with low doses of irradiation (70 Gy) had significantly ($P \leq 0.05$) higher levels of lycopene (Fig. 5) compared to fruit exposed to higher doses of irradiation (700 Gy) after the irradiation and storage. While early season fruit did not show storage or irradiation effects, late season fruit exhibited considerable decrease in lycopene content when fruit were stored up to 35 days. Moreover, initial lycopene content of late season fruit was significantly lower than early season fruit. These results indicate that maturity of grapefruit plays a crucial role in the carotenoid content of pigmented grapefruit. Grapefruit pulp attains maximum pigmentation in the early season but color deteriorates as the season progresses (Cruse et al., 1979). Grapefruit carotenoid, especially lycopene, appears to decline as the season progresses from October to May (Lee, 2000). Thus, it is possible that degradation of pigmentation initiated in late season fruit might have continued during the storage, thus, resulting in reduced lycopene content after 35 days of storage.

It is interesting to note that no significant differences ($P \leq 0.05$) were recorded between initial and final total carotenoid content in early season grapefruit except for fruit exposed to 400 Gy irradiation treatments (Fig. 6).

Limonin 17-β-D-glucopyranoside (LG) content of early-season fruit was not significantly affected by irradiation or storage (Fig. 7). However, higher LG content was recorded in fruit after 35 days of storage irrespective of the irradiation treatment in both early and late season fruit.

Late season grapefruit differed from early season fruit in their response to storage and irradiation. Storage and irradiation did not influence the β-carotene content of late season fruit after the simulated storage conditions. Late season fruit contained lower total carotenoids (β-carotene and lycopene) concentrations compared to early season fruit. It has been shown that some plants respond to oxidative stress induced by low temperatures by increasing the levels of antioxidants, such as carotenoids, and also by an increase in the activity of some antioxidative enzymes (Schoner and Krause, 1990; Walker and McKersie, 1993). It is interesting that late season fruit contained higher LG

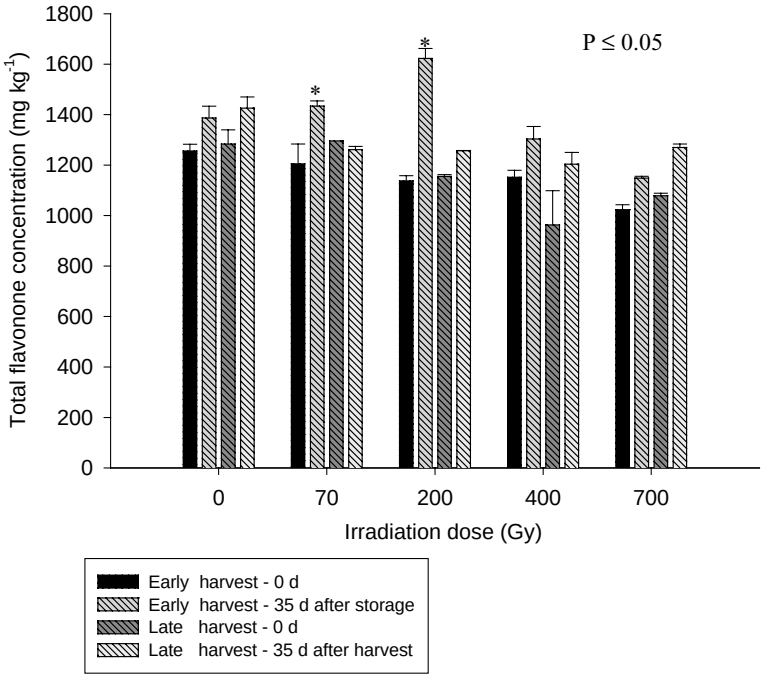

Fig. 3. Irradiation and storage effects on total flavanone content of early and late season 'Rio Red' grapefruit. Asterisk indicates significant ($P \leq 0.05$) differences between the mean values of 0 and 35 days after storage for early harvest fruit.

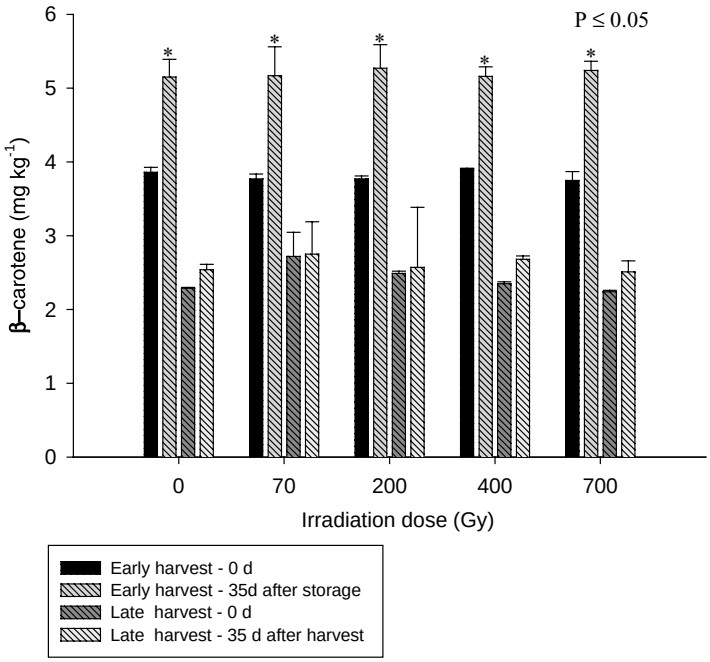

Fig. 4. Irradiation and storage effects on β-carotene content of early and late season 'Rio Red' grapefruit. Asterisk indicates significant ($P \leq 0.05$) differences between the mean values of 0 and 35 days after storage for early harvest fruit.

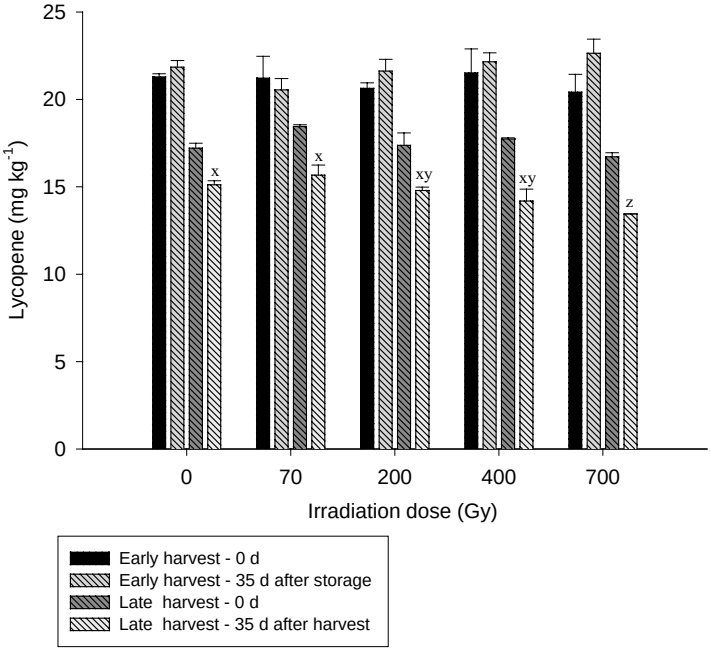

Fig. 5. Irradiation and storage effects on lycopene content of early and late season 'Rio Red' grapefruit. Same letter on the bar for late harvest fruit (0 day) indicates no significant differences at $P \leq 0.05$.

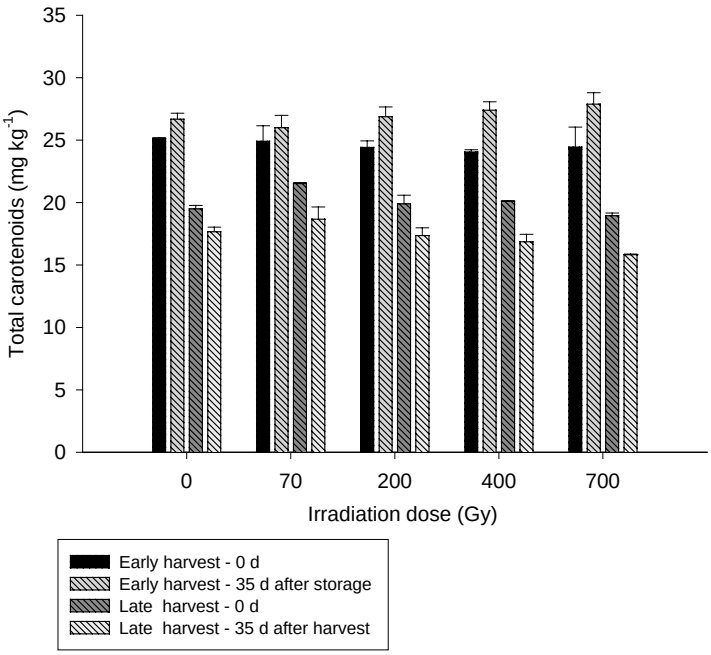

Fig. 6. Irradiation and storage effects on total carotenoid content of early and late season 'Rio Red' grapefruit.

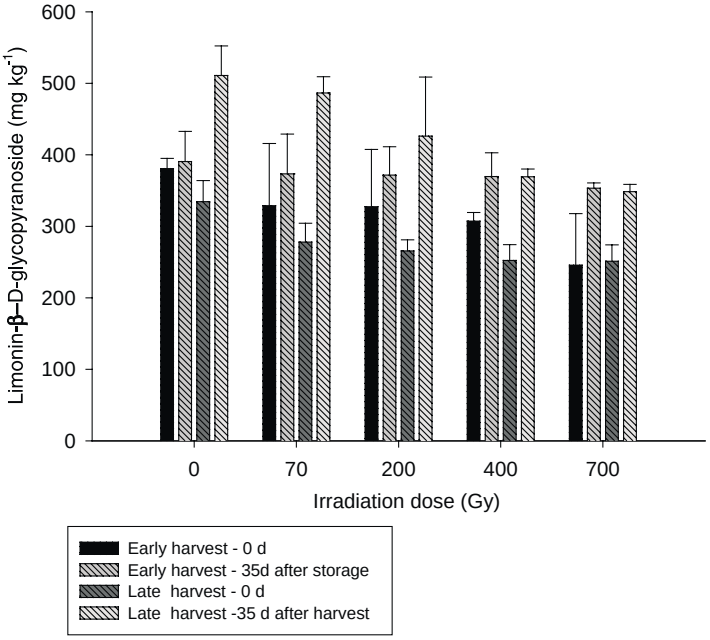

Fig. 7. Irradiation and storage effects on limonin-β-D-glycopyranoside content of early and late season 'Rio Red' grapefruit.

content than early season fruit. Very little information is available to relate oxidative stress and LG.

3.3. Changes in quality

Citrus fruit are important sources of Vitamin C in the human diet. Our study demonstrated that irradiation doses of up to 700 Gy had no significant effect on Vitamin C content of early-season grapefruit (Fig. 8). Previous studies suggest that loss of Vitamin C is minimal up to dose of 1000 Gy (Thomas, 1986). In general, doses adequate for quarantine purposes showed no significant loss in the Vitamin C content of several citrus fruit. In Spain, studies in clementine fruit irradiated up to 500 Gy along with hot water treatment and fruit stored at 17 °C for 3 weeks showed an increase in Vitamin C content (Abdellaoui et al., 1995). Moshonas and Shaw (1984) reported that 1000 Gy of gamma irradiation had no effect on Vitamin C content of grapefruit. It is possible that Vitamin C may not be the primary defense mechanism of fruit against the oxidative stress induced by gamma irradiation in early harvest. In general, 35 days of storage resulted in no significant changes in Vitamin C content of early season fruit.

Late season fruit exposed to an irradiation greater than or equal to 200 Gy caused a marked reduction in Vitamin C content after 35 days of storage (Fig. 8). A previous study reported that irradiation doses of more than 250–1500 Gy in grapefruit showed a decreasing trend in Vitamin C content (Yanez et al., 1990). Our results indicate that stress induced by irradiation above 200 Gy, coupled with low temperature stress may be harmful to the late season crop.

Quality characteristics such as soluble solids (%), TA or brix/acid ratios were not affected due to irradiation or storage of early season fruit (Figs. 9–11). Moshonas and Shaw (1984) found no significant differences in the quality of control and irradiated (300 Gy) grapefruit. However, late season fruit had lower soluble solids (%) and acidity values than early season fruit and the soluble solids/acid ratio after 35 days of storage were slightly higher than the initial ratios. Late harvest grapefruit exposed to irradiation (70–700 Gy) retained acidity better than the fruit not exposed to irradiation (0 Gy; Fig. 9). Initial soluble solids (%) was the lowest in the late season fruit exposed to 700 Gy irradiation; however, no differences among treatments were observed after storage.

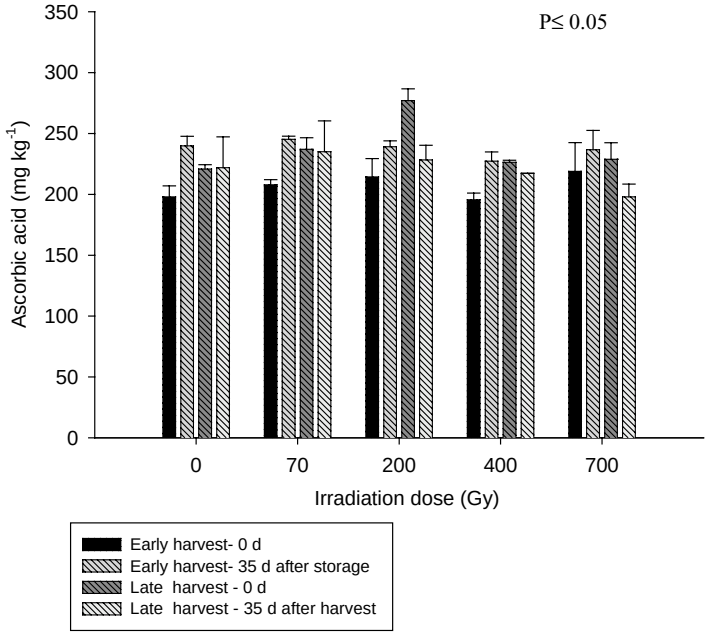

Fig. 8. Irradiation and storage effects on ascorbic acid content of early and late season 'Rio Red' grapefruit.

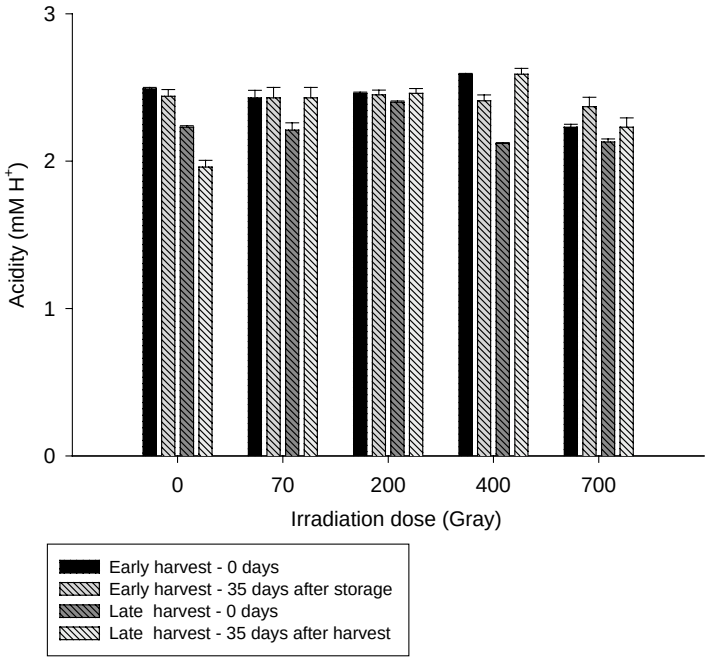

Fig. 9. Irradiation and storage effect on acidity of early and late season 'Rio Red' grapefruit.

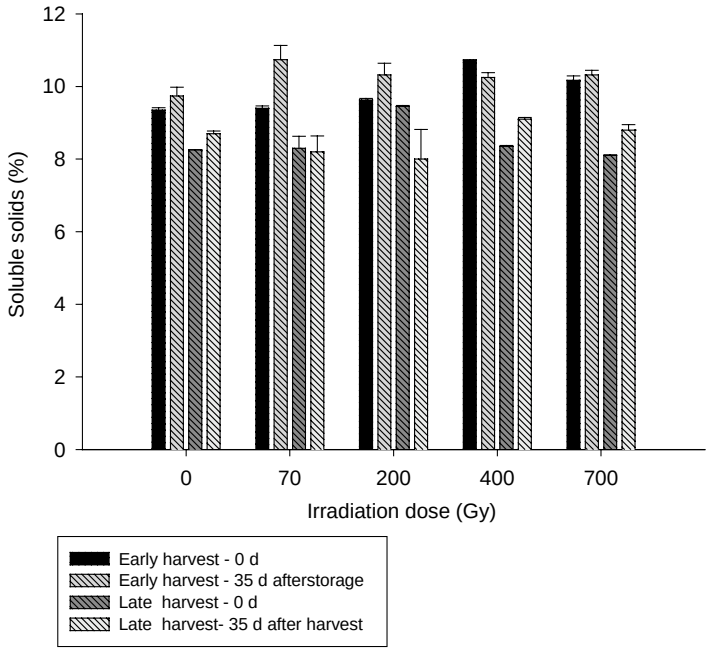

Fig. 10. Irradiation and storage effects on soluble solid (%) content of early and late season 'Rio Red' grapefruit.

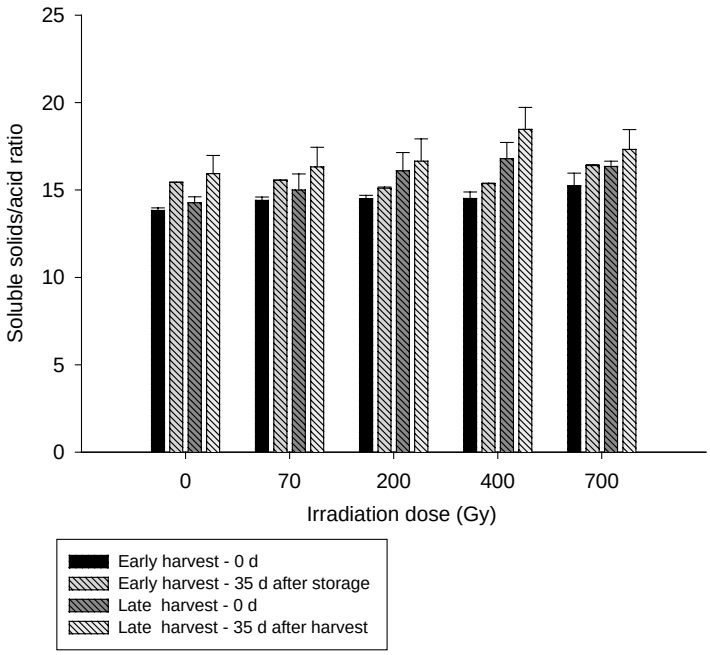

Fig. 11. Irradiation and storage effects on soluble solids/acid content of early and late season 'Rio Red' grapefruit.

Table 1
Gamma irradiation and 35 days of storage effects on appearance and flavor of 'Rio Red' grapefruit

Treatment	Early-season		Late-season	
	Appearance	Flavor	Appearance	Flavor
0	7.4 a	6.71 a	6.71 a	6.56 a
70	7.07 ab	6.03 ab	6.32 a	6.13 a
200	6.82 ab	6.07 ab	6.32 a	5.8 a
400	6.78 b	5.93 ab	6.16 a	5.76 a
700	5.75 c	5.46 b	6.4 a	5.76 a

Subjective scale: (1) extremely dislike and (9) extremely like. Data are means of 12 samples during each harvesting season. Means in a column followed by the same letter are not significantly different at $P \leq 0.05$.

Positive benefits in bioactive components may not have any practical significance, if irradiation used for quarantine purpose makes the fruit unmarketable. Preservation of typical sensorial qualities of irradiated fruit is one of the important requirements in terms of consumer acceptability. Sensory qualities such as appearance and flavor of early season grapefruit exposed to irradiation treatments at or below 400 Gy were comparable to the control after 35 days storage with the exception of the 700 Gy treatment, which was found to be detrimental (Table 1). Appearance rather than flavor of grapefruit was found to be more sensitive to irradiation. Irradiation had no significant ($P \leq 0.05$) effect on the sensory qualities of late season grapefruit. Nunez-Selles et al. (1986) reported negligible effects from irradiation (750 Gy) and storage treatments when organoleptic evaluation of juice was conducted. Trained panelist were able detect the difference in appearance of the whole fruit, flavor, taste and odor of the juice of California navel oranges irradiated at 300 and 600 Gy (O'Mahony and Goldstein, 1987). Irradiated tangerines from Brazil (Jobin et al., 1992) and irradiated Clementine from Spain (Abdellaoui et al., 1995) had acceptable flavor at 500 Gy; however, both hot water and irradiated fruit were not acceptable.

4. Conclusion

This study was conducted to provide information about the effect of irradiation on bioactive components and quality. The quality of grapefruit harvested in early-season was not affected by low dose or 35 days of storage; however, late-season grapefruit quality was adversely affected. Irradiation treatments and 35 days of storage influenced both flavanone and terpenoid contents of grapefruit; however, the latter was more prominent. In most cases, the optimal dose for enhancing bioactive constituents in early-season grapefruit was at or below 200 ppm, and for the late season crop, the optimal dose was 70 Gy. These results indicate that it is important to consider the harvest date when developing low dose irradiation quarantine techniques. Further studies on equilibrium between reduction in bioactive constituents due to gamma irradiation and de novo synthesis of these constituents at different time intervals during bioactive storage are essential to understand the mechanism.

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